Disinfection Protocol for Bat Field Research/Monitoring
U.S. Fish and Wildlife Service
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To minimize the potential for transmission of white-nose syndrome (WNS) while handling bats (both between handler and bats, between bats, and between handler and environment), these procedures are highly recommended. To date, WNS has been discovered in the northeastern US and mid-Atlantic states[1]. The U.S. Fish and Wildlife Service (USFWS) advises implementation of equipment decontamination protocols to reduce the risk of unintentional, human-assisted spread of WNS. In addition, we recommend that similar guidelines be used any time people handle wildlife to minimize potential disease-related impacts to wildlife and people. ***Please note that individual states/agencies may have additional permitting requirements above and beyond these general procedures.*** Additional restrictions apply for individuals conducting research in USFWS Region 3 - Ohio, Indiana, Illinois, Missouri, Iowa, Wisconsin, Michigan and Minnesota - either under a federal permit or Section 6 authorities as these states are currently unaffected by WNS. The requirements for Region 3 are posted at: http://www.fws.gov/midwest/Endangered/mammals/BatDisinfectionProtocol.html. These guidelines may be revised upon review of new information.

Any equipment that comes in contact with bats, individuals handling bats, or the environments where bats occur has the potential to be a vector for the spread of WNS. Examples include mist nets, harp traps, bat bags, wing biopsy punches, weighing tubes, rulers, clothing, and gloves.

Decontamination recommendations target the fungus *Geomyces* sp., which to date has been the most consistent pathogen recovered from bats exhibiting signs of WNS. Fortunately, many of the disinfectants/techniques tested for efficacy against the fungus are also suitable to kill other bacterial or viral agents should another causative agent of this disease be identified.

CAUTION: Disinfectant efficacy is based on application to hard, nonporous surfaces and the ability to prevent the regrowth of *Geomyces* sp. on artificial culture media. Tests are currently being conducted on porous fiber materials such as ropes and harnesses to determine disinfectant efficacy to kill the fungus on these substrates and their effects on gear integrity. The repeated use of disinfecting agents may compromise the effective use of vertical equipment; therefore, this equipment should be dedicated to one cave or not used at all.

Although a site may be affected with WNS, it should not be assumed that all individual bats within the site are infected or will become infected, and thus, care should be taken not to cross-contaminate specimens by lax handling methods. This is especially true if samples are to be submitted for diagnostic purposes.

Decontaminate all clothing, footwear, and gear prior to departing for a bat netting or cave outing if you did not decontaminate these items after last netting activity or exiting a cave. In affected and unaffected states, we ask that you not take gear into a cave if that gear cannot be thoroughly decontaminated or disposed of (i.e. if harnesses, ropes, or webbing cannot be decontaminated, we advise that you not enter caves or parts of caves requiring use of this gear).

In addition, only bring essential equipment used for bat netting and processing to a site, other non-essential items should be left home as they may contribute to spreading the fungus.

PROCEDURES:

Vehicles:

Do not work on live bats in vehicles. Vehicles used to transport equipment may harbor spores. Do all processing on vehicle hood or on a table away from the vehicle. The tailgate is not preferred since it is likely near netting equipment. A drawstring garbage bag should be placed at each site outside the field vehicle each night so all contaminated bags, gloves, wipes, etc., are contained. Dead bats should be placed in a sealed plastic container and placed inside a second bag or container handled only with clean gloves. This outer packaging layer is considered clean and uncontaminated and safe to transport inside the vehicle (preferably contained within a clean cooler).

Submersible Gear (i.e. clothing and soft-sided equipment):

- For clothing – Wash all clothing and any appropriate equipment in washing machine using the hottest cycle possible for material and conventional detergents. Laboratory testing has found Woolite® fabric wash to be the best surfactant for clothing. Rinse thoroughly, and then follow by soaking with sodium hypochlorite bleach (i.e. household bleach) solution diluted to 1 part bleach to 9 parts water in a tub or plastic container. Soak for 10 minutes, then rinse and air dry. If field projects necessitate extended efforts at remote locations, with no travel to new or additional sites, and daily washing or decontamination is not possible, then at the least, wash/decontaminate all clothing and other soft-sided equipment that has had direct contact with bats using the recommended procedures specified above.

- For other submersible gear (i.e. bags, gloves, nets, etc.) – Disinfect any equipment that can be submersed in a solution with an appropriate and compatible disinfectant such as sodium hypochlorite bleach (i.e. household bleach) solution diluted to 1 part bleach to 9 parts water in a tub or plastic container or $\geq$ 0.3% concentration of quaternary ammonium compounds (i.e. Sparquat 256, Lysol® All-purpose Professional Cleaner, or the antibacterial form of Formula 409®). Keep submersed for 10 minutes, then rinse and air dry.

Nets:

- Use separate sets between states known to be affected by WNS[1] and states currently unaffected. Realizing that some WNS affected states contain both affected and unaffected sites, under no circumstances should nets that have been used in an affected site be used in an unaffected site. Contact your state wildlife agency for updated information regarding WNS affected sites by visiting the following webpage http://www.fws.gov/offices/statelinks.html.

Bats should be kept in breathable holding bags rather than holding cages. To avoid cross-contamination of samples, it is imperative to keep bats separated using holding bags that are kept as clean as possible. Non-disposable holding bags should be used only once per night of field work and should be washed and decontaminated (following procedures above) and dried between nights of use. Disposable paper bags are also a convenient option for holding bats temporarily. Only one bat should be in a given bag, and that bag should not be reused for a new bat.

Disposable exam gloves should be worn over handling gloves and changed in between handling each bat. Disposable gloves should be one size larger than the handling gloves. Smooth leather gloves may be wiped down with a disinfectant (i.e. Purell®, Lysol® disinfecting wipes or alcohol wipes) in between handling bats. If only using leather gloves, each handler should have several sets of gloves to interchange in between handling bats. This allows time to effectively kill the fungus and for the disinfectant to completely dry. After each night of netting, remove heavy soil deposits from surface of bags and gloves, soak in an appropriate disinfectant, then dry completely.

For situations when gloves may hinder field work (i.e. transmitter attachment) and bats come in contact with bare hands, apply hand sanitizer with alcohol (i.e. Purell®) after handling each bat. Make sure it dries completely before handling the next bat.

Non-submersible Gear (i.e. hard-sided equipment):

- For non-submersible gear (i.e. bat processing equipment, mist net poles, harp trap frames and legs, folding chairs, etc.) – Disinfect any equipment that cannot be submersed by applying an appropriate and compatible disinfectant to the outside surface by using ≥ 0.3% concentration of quaternary ammonium compounds such as Sparquat 256, Lysol® All-purpose Professional Cleaner or the antibacterial form of Formula 409®, or use sodium hypochlorite bleach (i.e. household bleach) solution diluted to 1 part bleach to 9 parts water. Keep on surface for 10 minutes, then rinse and air dry.

- For boots – Boots need to be fully scrubbed and rinsed so that all soil and organic material is removed. The entire rubber and leather boots, including soles and leather uppers, can then be disinfected with an appropriate disinfectant such as ≥ 0.3% concentration of quaternary ammonium compounds (i.e. Sparquat 256, Lysol® All-purpose Professional Cleaner or the antibacterial form of Formula 409®) or sodium hypochlorite bleach (i.e. household bleach) solution diluted to 1 part bleach to 9 parts water. Keep on surface for 10 minutes, then rinse and air dry.

Use one of the disinfecting agents listed above to sanitize all equipment that comes into contact with a bat's body, including light boxes, banding pliers, rulers, calipers, scale, etc. Any instrument coming into direct contact with bat skin should be rinsed free of chemical disinfectant using clean water or physiologic (0.9%) saline. Clean items after handling each bat. If using containers to weigh bats, separate containers used to weigh tree bats from cave bats, do not place tree bats in the same container previously used for a cave bat. Containers used to weigh bats (film canisters, baggies, cardboard rolls) should be disinfected in between handling each bat.

Paper lunch bags can be used for holding and weighing individual bats, and can be immediately discarded after each use. Plastic baggies can also be used to line weighing containers, and bats can even be held in unsealed plastic bags during forearm measurements, reducing contact with wing rulers or calipers. Discard used bags after each bat. Disinfect gloves or discard disposable gloves after handling each bat.

Harp traps:

- Use separate traps between states known to be affected by WNS[1] and states currently unaffected. Realizing that some WNS affected states contain both affected and unaffected sites, under no circumstances should traps that have been used in an affected site be used in an unaffected site. Contact your state wildlife agency for updated information regarding WNS affected sites by visiting the following webpage http://www.fws.gov/offices/statelinks.html.

- In both affected[1] and unaffected states, we recommend that traps be cleaned nightly after use to remove any dirt/debris from wires/lines and bags. Following cleaning, all surfaces should be sprayed with one of the disinfecting agents listed above. Swab the bag with disinfectant and allow to dry completely (preferably in the sun) prior to the next use. Do not use equipment in an unaffected site following use in an affected site.

- We recognize that when working at a maternity colony using harp traps where regular bat to bat contact occurs, that some of the recommended decontamination procedures may not be practical. Therefore, we recommend checking the catch bag more frequently in order to reduce the amount of time that bats are in contact with each other and the bag. To reduce cross-contamination, the catch bag may be lined with a sheet of plastic and replaced with new plastic periodically or wiped down with one of the disinfecting agents above. Disposable gloves should be worn over handling gloves and swapped out regularly throughout the night, or frequently disinfected using Lysol® disinfecting wipes or alcohol wipes.

Cameras, Computers, and Other Electronic Equipment:

If possible, do not bring electronic equipment to a netting site. If practical, cameras and other similar equipment that must be brought to a site may be wrapped in plastic wrap where only the lens is left unwrapped to allow for photos to be taken. The plastic wrap can then be decontaminated by using Lysol® disinfecting wipes and discarded after use. If using plastic wrap is not practical, alcohol wipes or Lysol® disinfecting wipes can be applied directly on surfaces.

Wing Biopsies:

If collecting wing biopsies for any approved research studies on Federally threatened or endangered bats, use a new (unused) sterile punch for each bat. For other bats, punches may be reused, but only if they are still sharp enough to make clean punches. If there is evidence of fungal infection on any individual, use new punches. Be sure to completely sterilize recycled punches between bats by dipping the cutting end in alcohol. Pass the cutting end through a flame 3-4 times, and then allow the flaming punch to naturally extinguish, and cool completely. The

cutting board must also be disinfected between processing individual bats using one of the agents detailed above. Disposable, stiff cardboard squares (1 per individual) can be used as an alternate support for biopsy.

Notification of Signs of WNS

As a reminder, the white fungus is only one of the signs of WNS. We do not expect to find bats with fungus on them during the summer or fall, but bats could still be infected during these seasons. Other possible signs of WNS may be damage to wings and tail membranes in the form of lesions, flakiness or dehydrated skin, discolored spots/scarring, multiple holes, or tears to leading edge of membranes. We encourage the use of Reichard's Wing Damage Index (link below) for assessing bats. Please photograph any damage you observe and report it to the nearest U.S. Fish and Wildlife Service Field Office and your state agency that issued your bat handling permit within 24 hours.
http://www.fws.gov/northeast/PDF/Reichard_Scarring%20index%20bat%20wings.pdf

Important Note: These protocols are posted on the U.S. Fish and Wildlife Service Northeast Region website at: http://www.fws.gov/northeast/white_nose.html. We recommend that you visit the site at least once every six weeks to ensure that you are using the most recent protocol in your permitted activities.

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[1] **WNS Affected States:** Connecticut, Massachusetts, New York, Pennsylvania, Vermont, New Hampshire, New Jersey, West Virginia, and Virginia

Note: The listed WNS affected and adjacent states are current as of 6-9-09, please visit http://www.fws.gov/northeast/white_nose.html for the most updated information.

What is known about *Geomyces* sp. viability:

- The fungus survives exposure to mammalian body temperature (38°C/100°F) for at least 3 days, but does not remain viable after 8 days (W. Stone, NYSDEC, pers. communication 4/14/09).

- The fungus survives exposure to temperature (30°C/86°F) for at least 15 days. (W. Stone, NYSDEC, pers. communication 4/14/09).

- Short-term incubation of fungus at higher temperatures reduces the number of conidia present and alters the morphology of the hyphae which may not inhibit growth once returned to colder temperatures (W. Stone, NYSDEC and D. Blehert, USGS NWHC, pers. communication 4/14/09).

- Clothes dryer heat treatment (49°C/ 120°F) alone increases fungal spore germination and does not kill the fungus (H. Barton, NKU, pers. communication 4/22/09).

What kills the *Geomyces* sp. fungus:

Method	Conditions	Kill Time	Source	Cautions*
Disinfectant				
5.25% Chlorine bleach	10% bath solution (1 part bleach: 9 parts water)	10 min	Over the counter	Inactivated by organic material, detergents; corrosive to metals; produces toxic gas if combined with ammonia; skin irritant
Lysol® Professional Antibacterial All Purpose Cleaner	1:128 bath solution (1 oz per 1 gal water)_	10 min	Janitorial supply	Corrosive; skin & eye irritant
	1:64 bath solution (2 oz per 1 gal water)	5 min		
Sparquat 256	½ oz per 1 gal water	10 min	www.chemsearch.com	May require license to obtain; requires special disposal methods
Promicidal™	1:128 bath solution (1 oz per 1 gal water)	10 min	www.chemsearch.com	May require license to obtain; requires special disposal methods
Grenadier™	1:64 bath solution (2 oz per 1 gal water)	10 min	www.chemsearch.com	May require license to obtain; requires hazardous waste disposal methods
	1:32 bath solution (4 oz per 1 gal water)	5 min		
Formula 409®	At least 0.3% concentration	10 min	Over the counter	
Woolite®	Refer to product label		Over the counter	
Dawn® antibacterial hand soap	Refer to product label		Over the counter	
Purell®	Refer to product label		Over the counter	
Lysol® disinfecting wipes	Refer to product label		Over the counter	

70%-95% ethanol	Undiluted bath	2 min	Lab supply distributor	Flammable, skin irritant
Temperature				
Dry heat	110°F/ 43°C	12 hr	Oven, incubators	
	165°F/ 74°C	15 min		
	175°F/ 79°C	5 min		
	180°F/ 82°C	5 min		
Sterilization				
Steam autoclave	121°C; 15 psi	15 min	Laboratory or hospital settings	
Gas sterilization	Ethylene oxide	16-18 hr	Only available at hospitals	
Flame sterilization	Alcohol & open flame	15-20 sec		Fire hazard; burn injuries

* Effects of different decontamination methods on the integrity of caving equipment are currently being tested.